NOTES ON THE BACTERIOLOGICAL ANALYSIS OF WATER.

BY L. H. PAMMEL.

The recent epidemic of typhoid fever at the college is of interest to us and especially the methods now in vogue with reference to the examination of water for various organisms. During the recent epidemic and previously the well waters in the vicinity of Ames as well as the college water supply were examined at various times. An examination has also been made of water coming from wells of the parties who have furnished milk to the college. It should be stated here that this report is not completed owing to the fact that some of the species have not been sufficiently determined. From the nature of the case it requires a great deal of patient and careful work to run out the different species, so that the biological examination was not completed. Thanks are due to Mr. F. W. Faurot, Mr. A. D. McKinley, Mr. H. H. Thomas, Miss Nellie Nicholas, Miss Estella Paddock, and Mr. L. R. Walker for assistance in carrying out this work.

In the paper on the Iowa State College Sewage Disposal Plant will be found a brief note on the water of the deep well previous to this spring. Examinations have been made from time to time, and as a result of our work, we found that the water during the winter months varied from no bacteria to 50 per cubic centimeter, thus showing an unusually good supply of water.

A Marston, J. B. Weems and L. H. Pammel. The Iowa State College Sewage Disposal Plant and Investigations. Proc. Ia. Engineering Soc. 1900. Contr. Ia. State Coll. Argrl. & Mech. Arts. 1:19.

BRILEY SHALLOW WELL.

Depth, 45 feet; 18-inch glazed tile, cemented at the joints, covered with boards on top. The well has not been used since October 20th.

DATE.	GAS TEST.	Total number bacteria per CC.	GELATINE.		Agar.	LITMUS AGAR.
			Lique-fy-ing.	Non-lique-fying		
October 17th.....	Present	18,000	.	... .	Used.	Some acid- pro-ducing germs.
October 18th.....	....	12,000	.....	.... .	Used.	
October 25th ...	First pumping..... ...	6,000	.... .	.....	Used.	
October 25th	One-half barrel pumped....	1,440	....	.... .	Used.	
October 25th	One barrel pumped	2,000	.....	.... .	Used.	
October 25th	1½ barrel pumped	2,400	..	.....	Used.	
October 29th ...	Present	125		125		

BRILEY DEEP WELL.

Depth, 185 feet; 2-inch pipe and casing.

October 17th	....	60	.....	.....	Used.	No acid-produc-ing germs.
October 17th	None	...	30			
October 18th	None	30				
October 27th	....	30	.....		Used	
October 29th	... .	30				

PRITCHARD WELL AND TANK—WELL.

Depth, 170 feet; 3-inch casing well and inside a 2-inch pipe.

October 18th	....	20				
October 22d..... .	.....	30	.. .	30	Used.	
October 29th.....	.....	20	....	20	.....	Non-acid-pro-ducing.
October 18th	....	60	20	40		

TANK.

Open tank used for watering stock, above well.

October 29th.....	.....	40				
October 29th.....	3½ cc.....	160				
October 31st.....	.....	225	.....	.....	225	
October 29th.....	3½ cc.....	.....	.....	.....	.....	Acid reaction.

PETERSON WELL AND TANK.—WELL.

Depth, 185 feet; 120 feet down to cylinder. Cased. Two inch iron, with about four inch of casing. Located two miles north of Ontario.

DATE.	GAS TEST.	Total number bacteria per cc.	GELATINE.		Agar.	LITMUS AGAR.
			Liquefying.	Non-liquefying.		
October 27th	7 cc					
October 18th		150			150	
October 18th		170				
October 18th		1,500			1,500	
October 27th		3,360	3,360			
October 29th		9,000	1,800	7,200		
October 18th		80				80 Non-acid.

TANK.

Open tank for watering stock.

October 18th	3 cc	25			25	Acid producing
October 29th	Acid	2,600	360	2,240		
October 31st	10 molds	4,200			4,200	100 acid.
October 18th					320	220 non acid.

SKELTON WELL.

Thirty-five feet deep, ten inch casing.

October 18th		30			30	Non-acid.
October 18th		10				
October 27th	3 cc					Acid.
October 31st		200			200	
October 27th	2 molds	90				
October 27th	No. 2	633			633	

RIVER WATER.

DATE.	SKUNK RIVER WATER.		DATE	SQUAW CREEK WATER.	
	Total number germs per cc.	Medium used agar.		Total number germs per cc.	Medium used agar.
April 30th	1,800		May 19th	300	
May 7th	1,800		July 2nd	11,200	
May 9th	916		August 8th	16,200	
May 19th	1,800		August 8th	8,520	
July 6th	27,000		October 4th	2,400	

Investigations carried on with the water supply of various wells in the vicinity of Ames by Messrs. McKinley and Thomas and Mr. Faurot gave the following results:

FAUROT'S WELL.

DATE.	Number germs per cc.	REMARKS.
April 23d.....	1,600	
April 23d.....	4,500	
May 22d.....	9,360	
May 29th.....	9,480	
July 6th.....	220	Collected after a rain
Average.....	5,032	

OTIS HOUSE WELL.

May 7th.....	80	Collected without ice.
May 21st.....	3	Collected without ice.
May 28th.....	200	Collected without ice.
July 2d.....	54,000	Indication of something in pipes.
August 8th.....	120	Indication of something in pipes.
August 8th.....	None	After pumping 15 minutes, collected with ice.
October 4th.....	120	With ice—first pumping.
October 4th.....	360	With ice—after pumping.
October 23d.....	3,000	First pumping—no gas.
October 23d.....	2,400	Second pumping—no gas.
Average.....	6,028	

LABORATORY TAP.

May 7th.....	None.	Poured immediately.
May 21st.....	None.	Poured immediately.
October 4th.....	360	Poured immediately.
October 17th.....	520	Poured immediately.
October 17th.....	700	Poured immediately.
November 6th.....	80	Poured immediately.
Average.....	276	

PARSON'S WELL.

May 7th.....	3,600	Well full.
May 21st.....	Failure.	Well full.
May 28th.....	1,300	Well full.
July 2nd.....	90	With ice. First pumping. Very little water in well.
August 8th.....	150	With ice. First pumping. Very little water in well.
August 8th.....	170	With ice. Second pumping. Very little water in well.
October 23d.....	50	Without ice. Second pumping. No gas.
October 23d.....	380	Without ice. First pumping.
Average.	643	

ILLSLEY'S WELL.

DATE.	Number germs per cc.	REMARKS.
May 7th	8,000	
May 21st	Failure.	
May 28th	600.	
July 2d	1,200	
August 8th	590	Without ice. First pumping.
August 8th	220	With ice. Second pumping.
October 23d.	80	No gas. Second pumping.
October 23d.	800	First pumping.
Average	1,642	

WELL AT HOUSE NEAR BRICK YARD.

May 21st	300	
July 2d	330	
August 8th	10,800	First pumping.
August 8th	7,800	With ice. Second pumping.
October 4th	1,400	With ice. First pumping.
October 4th.	5,400	With ice. Second pumping.
Average	4,338	

CREEK WATER.

May 19th	300	
July 2d	11,200	
August 8th	16,200	Without ice.
August 8th	8,520	With ice.
October 4th	2,400	With ice,
Average	7,724	

OLSEN'S WELL.

May 28th	10	
August 8th	60	With ice. Wind mill in operation one-half day.
August 8th	350	Wind mill in operation one-half day.
October 4th	600	With ice. First pumping.
October 4th.	120	With ice. Second pumping.
October 23th	620	Without ice. First pumping.
October 23th	240	Without ice. Second pumping. No gas.
Average	286	

FOUNTAIN WATER IN PARK, STORY CITY, IOWA.

October 7th	4,500	Without ice. Poured in laboratory. No gas.
October 13th	20	Poured immediately.

HIGH SCHOOL, STORY CITY, IOWA.

October 7th	400	Collected without ice. No gas.
October 13th	440	Poured at well.

HENRYSON'S WELL, STORY CITY, IOWA.

DATE.	Number germs per cc.	REMARKS.
October 7th.....	280	Collected without ice Produced gas.
October 13th.....	230	Poured at well.

HYDRANT, STORY CITY, IOWA.

October 7th.....	520	Without ice. No gas.
October 13th.....	30	Poured at hydrant.

C. & N. W. WELL AT WEBSTER CITY, IOWA.

October 6th.....	310	Without ice. Gas.

A. J. HAVILAND'S WELL, FORT DODGE, IOWA.

October 5th.....	150	Without ice. 30 moulds.

WILL HAVILAND'S WELL, FORT DODGE, IOWA.

October 5th.....	5,400	Without ice.

The records kept by Miss Nicholas were as follows:

MUNN'S WELL.

May 5th	570	Agar used.
September 24th....	300	Agar used.
October 11th.....	80	Agar used.

PAMMEL'S WELL.

September 9th.....	1,300	Agar used.
August 11th.....	400	Agar used.
September 27th....	510	Agar used.

BUDD'S WELL.

May 5th.....	50	Agar used.
September 27th...	40	Agar used.
October 8th.....	30	Agar used.
October 27th.....	20	Litmus agar used. Non-acid producing.

REED'S WELL.

DATE.	Number germs per cc.	REMARKS.
May 17th	2,500	Agar used.
May 31st.....	1,200	Agar used.
September 19th...	700	Agar used.
October 27th	.....	Litmus agar used. Acid and non-acid.

MILLER'S WELL.

May 17th	270	Agar used.
May 31st.....	400	Agar used.

PAXTON'S WELL.

May 17th	1,900	Agar used.
September 19th....	1.300	Agar used.
September 27th,...	2,400	Agar used.

HARDIN'S WELL.

May 31st.....	30	Agar used.

LINCOLN'S WELL.

May 5th.....	300	Agar used.)
May 31st.....	400	Agar used. } No gas at any time.
September 27th....	100	Agar used.)

HUNT'S CISTERN.

May 17th	150	Agar used.

HOOVER'S SPRING.

May 17th.....	2,400	Agar used.
October 27th	40	Litmus agar used. Non acid producing.

The following are the results of Miss Nicholas of examination of samples, the second after discarding a few pails-full. The medium used was ordinary agar.

DATE.	Well	First pumping.	Second pumping.
September 9th	Lincoln...	460	330
September 9th	Munn.....	240	230
October 8th	Budd.....	30	20
October 8th	Lincoln...	180	170
October 8th	Reed.....	1,700	1,600
	Kinkade..	2,800	6,000

The Kinkade well is very shallow and the second sample was collected after several barrels of water had been pumped out, therefore the much greater number of bacteria in the second sample may be due to sediment.

All of the shallow wells examined contained gas-producing germs. The Paxton well produced 30 cc. of gas in the fermentation tube, 10 cc. of which was CO_2 and 20 cc. CH_4. The Reed well produced 100 cc. of gas (40 cc. CO_2 and 60 cc. CH_4). The water from the Kinkade well produced a very great amount of gas.

The Briley Shallow Well.—In conjunction with Dr. Weems and Mr. McKinley on another occasion the writer collected samples of the water at the Briley well, and later Mr. Faurot also collected this water twice. The second time when Mr. Faurot collected these samples we got an unusually large number of germs per cubic centimeter. That collected by the writer on October 17 had 18,000 and that by Mr. Faurot had 6,000. It is worthy of note in this connection that the samples collected by myself on October 17 contained 18,000 germs per cc., that in one of the samples collected by Mr. Faurot on October 25, the number of germs had diminished very materially, the largest number found was 6,000. On October 29 the highest number obtained was 125 per cc.

In regard to the last plates poured it is a singular fact that but a very small development occurred, and this is strange since we had such an unusual development before running from 6,000 to 18,000 per cubic centimeter.

In regard to the condition of the well it looks as though the water could easily have drained off from the surface, but nevertheless upon removing some of the boards from the top of the well I found that the water might easily have entered between the cracks of some of the boards. In fact I found moisture on the inside on the upper tile, showing the water had run down. One can readily see how *B. coli-communis* or other foreign organisms could get into the water. Gas was produced in one tube poured by Mr. Faurot and a slight amount in another. In this case we made the usual test. We also obtained gas from the first plates that I poured.

The samples collected on October 29 were kept for forty days in the laboratory and then were examined by Mr. McKinley and Mr. Thomas with the following results:

WELL.	Depth.	No. of germs.
Briley Shallow Well	45 feet.	200
Briley Deep Well	185 feet.	20
Laboratory tap. Same source		10
Kitchen Tap		None.
Skelton's Well	35 feet.	30
Peterson Deep Well	185 feet.	340
Peterson's Trough		1.000
Pritchard Well	170 feet.	30

Various species were found. Some of these have been excluded as having no connection with *Bacillus typhosus* or *B. coli-communis*. On the other hand there are a number of species that belong to the typhosus group culturally so far as has been carried out. Our work was interrupted although cultures of all of the species were made and placed away for further study. Fire destroyed the entire laboratory so no further study can be made.

One peculiar pearly white Bacillus developed in considerable quantity, in fact at least three-fourths of the colon-

ies belonged to this species. This Bacillus though actively motile had none of the cultural peculiarities of *B. typhosus*. Two species are quite commonly found in surface waters, namely the *B. cloacæ* first detected by Jordan in sewage.

I am inclined to think that both *B. coli-communis* and *B. cloacæ* occurred in the Briley shallow well, but the definite separation was not carried far enough to determine this point to my satisfaction, though Dr. Eli Grimes states *B. coli-communis* was found.

THE COLLEGE WATER SUPPLY.

It is certainly worthy of mention in this connection that all of the species found in the college water supply in the tank are non-liquefying, and the fact that gas was found on one occasion does not argue that the college water supply was contaminated. The simple fact that the species here found did not produce gas in the proportion given for *B. coli-communis*, namely, of two parts of H. to one part of CO_2, but represented by formula one to two. It is also a significant fact that morphologically none of the species found indicated either *B. coli-communis* or *B. typhosus* in the college water supply.

Of the oft-repeated statement that sewage contamination might have occurred, I wish to state that the writer, together with Professor Marston, climbed to the top of the tower and investigated conditions, and everything was found in its usual good condition. There was certainly no indication of growth of algæ on the water, nor were there any indications of other filthy conditions. In fact, the water, and everything connected with it, seemed to be in an ideal state.

The statement has also been made that owing to the fact that the college at different intervals used the supply from the spring, and in this way became contaminated. An investigation made of the college spring water, as well as the different hydrants and cisterns, those of Professor Stanton, Professor Curtiss, and the old Sexton well, indi-

Experimental Investigations St. Brd Health, Massachusetts, **1889-1890**: 836, and later found by Moore to be widely distributed in the soil.

Russell and Bassett. Trans. Amer. Pub. Health Asso., **25**.

cate unusually good water, with the exception that in the Curtiss well and the Sexton well gas was produced, but this undoubtedly came from the surface soil. The spring water showed no gas whatever, nor was any obtained from the hydrant which was next to the spring. The samples and plates were carefully plated.

BACTERIA FOUND IN OTHER WATER SUPPLIES.

We have found quite commonly in all of our waters the *B. liquefaciens-fluorescens.* The *Tyrothrix* of Duclaux is certainly also common. Most attention has been given to the chromogenes. The common genera of Bacillus and Micrococcus were represented, and of the these the Micrococci were found more frequently than the Bacilli of these *Micrococcus roseus-flavus*, Hefferan, *M. agilis*, A. Cohn, and others were found.

BACILLUS TYPHOSUS IN WATER.

Now, as to the relative vitality of *Bacillus typhosus* in water; many determinations have been made, and it would not be strange if the *Bacillus typhosus* should not be found in water.

It is usually held by sanitarians that water is the most frequent source of infection. The evidence of *B. typhosus* in water, in most cases, is circumstantial; but I recall a case where Dr. Ravold found it in Mississippi river water, and bacteriological journals report cases of its occurrence in wells and streams, but the reported findings of the organism under such circumstances are not numerous. It is very evident that the typhoid fever bacillus will not grow in the ordinary media with other pathogenic organisms, nor are the special media much more satisfactory. It is evident from the results obtained from several investigators that not much can be expected from the organism after four weeks. It is certain that the typhoid fever organism will not multiply freely in water.

MILK AS A SOURCE OF CONTAMINATION.

As to the bacteria found in the milk supply, an investigation has been made, but this work was not completed,

owing to the destruction by fire of all of our cultures. We found present in the milk a large number of chromogenes, but none of these, of course, can be referred to, or are in any way related to the typhoid fever bacillus. On the other hand, we did find *B. coli-communis*, but it does not necessarily follow that the *B. coli-communis* comes from human dejecta, as this organism is very commonly found in connection with cow stables, and the organism being found quit frequently in the intestinal tract of animals as well as man. Therefore this cannot be considered to be the cause, nor as an argument against the use of milk. This work, however, was not completed, and hence a final statement cannot be made.

COMPARISON WITH THE SEWAGE BACTERIA.

The results of the work carried on on the College Sewage Plant show the following conditions with reference to the purification, and it is of interest to compare these results with the water obtained from the Briley well. It will be seen that in every case, excepting the last one, that the Briley well contained many times more organisms than the effluent of either filter bed.

DATE	From	Air	Water	Manhole	Tank	Effluent
September 1st	W. E.	90 degrees	75 degrees			960
September 2d	W. E.	72 degrees	71 degrees			2,400
September 3d	E. E.	62 degrees	73 degrees			2,100
September 4th	W. E.	63 degrees	72 degrees			390
September 5th	E. E.	82 degrees	72 degrees			230
September 5th	Tank		68 degrees		242,400	
September 5th	Manhole		68 degrees	1,212,000		
September 6th	W. E.	83 degrees	72 degrees			1,800
September 7th	W. E.	82 degrees	74 degrees			460
September 8th	W. E.	90 degrees	74 degrees			230
September 9th	E. E.	87 degrees	74 degrees			310
September 10th	Tank				424,200	
September 10th	Manhole		62 degrees	1,363,000		
September 10th	E. E.	68 degrees	72 degrees			210
September 11th	E. E.	69 degrees	73 degrees			440
September 12th	W. E.	70 degrees	74 degrees			110
September 13th	W. E.	84 degrees	72 degrees			1,200
September 14th	W. E.	85 degrees	74 degrees			480
September 15th	W. E.	55 degrees	74 degrees			100
September 16th	E. E.	65 degrees	72 degrees			320
September 17th	E. E.	68 degrees	70 degrees			3,000
September 17th	Tank		64 degrees		484,600	
September 17th	Manhole		68 degrees	696,600		
September 18th	W. E.	50 degrees	64 degrees			460
September 19th	W. E.	66 degrees	65 degrees			340
September 20th	E. E.	72 degrees	67 degrees			420
September 21st	W. E.	71 degrees	67 degrees			340
September 22d	E. E.	79 degrees	66 degrees			480

From September 23d to September 28th, inclusive, the sewage effluent pipe was under water, hence no samples.

DATE.	From	Air	Water	Manhole	Tank	Effluent
September 29th	E. E	69 degrees	64 degrees			980
September 30th	W. E	68 degrees	64 degrees			460
October 1st	W. E	72 degrees	65 degrees			360
October 1st	Tank		67 degrees		568, 400	
October 1st	Manhole			896, 600		
October 2d	W. E	80 degrees	67 degrees			1, 200
October 3d	E. E	75 degrees	67 degrees			360
October 4th	W. E	81 degrees	67 degrees			1, 800
October 5th	E. E	80 degrees	67 degrees			450
October 6th	E. E	72 degrees	68 degrees			1, 200
October 7th	W. E	63 degrees	68 degrees			2, 100
October 8th	E. E	40 degrees	68 degrees			1, 800
October 8th	Tank		62 degrees		260, 000	
October 8th	Manhole		61 degrees	1, 333, 200		
October 9th	W. E	63 degrees	70 degrees			2, 400

From 10th to 13th, inclusive, the beds were being cleaned and the sewage was turned directly into the creek from the tank.

October 14th	W. E	63 degrees	63 degrees			360
October 15th	W. E	63 degrees	63 degrees			210
October 15th	Tank		63 degrees		1, 2r2, 000	
October 15th	Manhole		64 degrees	*		
October 16th	W. E	60 degrees	62 degrees			120
October 17th	W. E	55 degrees	62 degrees			120
October 18th	E. E	63 degrees	61 degrees			130

* Too thick to count. Estimated at 5, 000, 000.

CONCLUSION.

It may be stated that so far as the analysis show the college water supply may be considered excellent. It is true that in a number of instances more organisms were found than at other times, but an examination made from time to time shows that the number is not unusually large, and on the whole that we may consider our water supply practically pure, and I should also state that the water from the spring supply is unusually good. We should bear in mind that the failure to find the typhoid fever bacillus in the water supply or milk of the Briley well is not at all surprising. It is a well known fact that the saprophytic species grow so readily in the nutrient media that the typhoid fever bacillus has not the same chance to grow. The same may also be said with reference to milk, only here we are dealing with such a large

number of species that it would be a mere accident to discover the organism. As said heretofore it seems to me to be reasonable that the milk formed a favorable medium for the growth of the organism, and be it specially remembered that Mr. Briley, from his own testimony, failed to wash the cans with boiling water as should have been done. The milk cans could easily have been contaminated, and the failure on his part to wash the cans, it seems to me, made it not only possible but probable that these germs propagated in the milk.

A comparison of the water of the Briley well and the college effluent shows that the Briley well had a greater amount of contamination than the college effluent from the sewage filter beds.